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THE TOXIC EFFECTS OF CERTAIN HEAVY METALLIC IONS ON SEWAGE SLIMES

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THE TOXIC EFFECTS OF CERTAIN HEAVY METALLIC IONS

ON SEWAGE SLIMES

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

THE TOXIC EFFECTS OF CERTAIN HEAVY METALLIC IONS ON SEWAGE SLIMES

CHAPTER I

INTRODUCTION

The relative ease and efficiency of using biological treatment for the degradation of domestic sewage waste water leads to a search for methods of applying these processes to industrial waste waters. Large amounts of toxic materials in some industrial waste waters have resulted in reduced efficiency or even a complete cessation of biological activity.

Research concerning the toxic effects of the constituents of industrial waste waters has been concomitant with the problems presented by the increasing demands for better water. Increasing population and the creation of giant industrial complexes have produced an environment in which there is little opportunity for streams and rivers to recover their quality after receiving waste waters. Modern technology has created new products and wastes and has further demanded the improvement of existing products. The net effect has often been the creation of wastes even more resistant to biological treatment.

The metal fabricating and plating industries have a long history of producing quantities of waste waters containing solutions of metallic ions toxic to stream biota. In some cases both the metallic and

non-metallic parts of the dissolved compounds have been toxic to the microorganisms essential to waste treatment. Cupric sulfate is an example of such a compound. Rinse solutions and plating baths have been the main contributors of metallic ions to industrial wastes. For example, 26 percent reduction in BOD (biochemical oxygen demand) removal due to 2 mg/l of chromate ion in the influent waste stream was reported at a Fairfax, Connecticut sewage disposal and treatment plant. (1)

The Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio has recently completed a series of investigations into the toxic effects of several metallic ions on models of activated sludge and sludge digestion systems. (2) Other studies have been made of the effects of metallic ions on the ecological balance of streams, rivers, and lakes. (3) Tolerance levels of different species of aquatic life to dissolved metals have been determined in some cases. Chromate wastes in concentrations as low as 0.03 mg/l are toxic to Daphnia magna, an important fish food in streams and an index of fish toxicity. (4) As little as 0.1 mg/l of chromate in drinking water exhibits a toxic effect on humans. (5) The United States Public Health Service recommends a concentration of chromate no greater than 0.03 mg/l as a criteria for potable water. (6)

In any biological system there are interactions between the inhabitants and the environment. Toxic materials present in the environment will tend to inhibit biological activity. The toxic materials will in turn be removed from the environment by the biological activity or the by-products of this activity. Although the two interactions are undoubtedly dependent upon one another, the investigation reported here attempts to study only the toxic effect of some of the important metallic

ions found in industrial waste waters. The ions under investigation are hexavalent chromium, divalent copper and nickel, in the forms of potassium chromate, copper sulfate, and nickel nitrate. These metallic ions are of particular interest because they are the ones most frequently found in industrial plating wastes. The study of the rate of removal of these metallic ions by the microorganisms found in a waste water treatment plant is left to future research.

Industrial waste waters may arrive at a treatment plant continuously or in slug doses. In general, slug doses tend to contain the greater concentrations of toxic materials. The damaging effects of massive slug doses of toxic wastes are often eliminated by slowly releasing the wastes from holding tanks. Because of equipment costs for such an installation many small industries dump their wastes directly into the sewers; these wastes arrive at the treatment plant as slugs, oftentimes unexpected and undetected. The study of slug doses of toxic materials is thus justified by this practice.

In the early 1950's studies of the waste waters generated by U. S. Air Force Maintenance Installations were begun. (4) Some of these studies were concerned with plating wastes from jet engine maintenance shops and the chromate wastes from air conditioning cooling towers. To evaluate effects of these wastes on sewage slimes a means to simulate biological treatment had to be devised. This was done by Reid, et al. (7) A laboratory arrangement for growing attached slimes simulated a trickling filter. This model consisted of small glass drums rotating in a nutrient broth containing a radioactive tracer. The rate of removal of radiophosphorous by the attached slimes, as measured with a Geiger-Mueller tube and scalar, was correlated to the growth of the slime

producing microorganisms. Decrease in growth rates due to the presence of metallic ions was interpreted as evidence of toxicity. These studies indicate the existence of a quantitative relationship between growth rates and concentrations of metallic ions present.

The current study attempts to corraborate previous results and depict and explain the effects of a substrate containing known amounts of metallic ions on conventional waste treatment processes.

Literature Survey

Reid, et al. (8) investigated the effects of metallic ions fed in continuous doses to attached slimes. The slimes demonstrated an ability to acclimate to the metallic ions. Low concentrations of chromate ions, 2 and 5 mg/l, had a stimulating effect. This effect has been noted by others. (9) Concentrations as high as 60 mg/l of chromate ion had no detrimental effect on the attached slimes. Shock effects were noted when unacclimated slimes were first placed in substrates containing the metallic ions.

Reid (4) in a study of industrial waste waters produced at Tinker Air Force Base, Oklahoma, determined the toxicity of copper, hexavalent chromium, nickel and other metals using slug doses. Concentrations as high as 3 mg/l of the various metals, singly and in combination, were studied with reductions in efficiency up to 73.5 percent occurring, as shown in Table 1. Here "K" is a measure of the growth rate of the attached slimes and will be fully explained later. As can be seen from Table 1, small concentrations of metallic ions produce a large toxic effect on attached slimes.

This study is concerned with this decrease in efficiency or growth

rate due to the initial shock upon receiving slug doses of metallic ions. Reid (4) studied slug concentrations as high as 3 mg/l; the current study involves concentrations up to 10 mg/l in slugs.

TABLE 1

PERCENTAGE DECREASE IN "K" VALUES IN THE PRESENCE OF METALLIC IONS

Concentration (ppm)	Chromate	Cadmium	Copper	Nickel	Aluminum	Silver
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.50	2.30	50.50	23.00		31.00	40.30
1.00	21.80	63.00	25.30		45.00	49.50
1.50	50.50	73.50	54.00	25.30	52.00	61.00
2.00				38.00		
3.00				57.50		

Researchers at the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio studied the effects of heavy metals, chromium, copper, nickel, and zinc on biological treatment processes and compared their results with field studies on operational treatment plants. (10, 2, 11, 12, 13) Most of their work was done with continuous dosages of the metallic ions as opposed to slug or batch doses. They used systems which had been acclimated to the toxic ions being studied--copper, chromate, nickel, and zinc. Individual studies were made of the various ions. Results indicate that initially toxicity increases with concentration, but soon levels off to form a plateau with little further decrease in toxicity; the plateau ends at much higher concentrations. As seen in Figure 1, the toxic effect increases sharply again at the end of the plateau. The authors gave no indication of the range of values for this

figure, but the toxic effect is reported as the percentage reduction in BOD removal by the system under consideration. There is no evidence of a plateau in the data given in Table 1 but concentrations go only as high as 3 mg/1. Perhaps the plateau begins at some higher concentration.

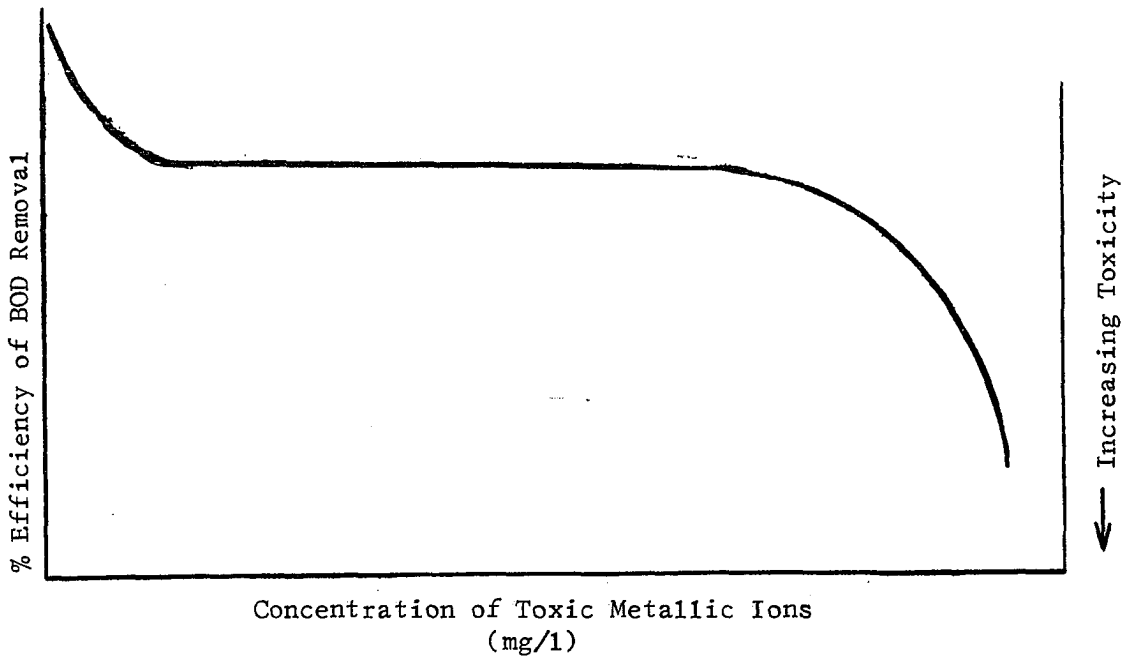


Figure 1.--Reduction in BOD Removal

Over 90 percent of the zinc introduced to the system was converted to an insoluble form by the chemical and physical actions of the sewage and thus was removed as a toxicant. The system acclimated itself to the remaining soluble zinc. This acclimation was evident for concentrations of zinc cyanide as high as 20 mg/1. The microorganisms could not acclimate to doses of zinc sulfate as low as 10 mg/1. Slug or batch doses of all metals as cyanide complexes were also studied; cyanide toxicity was overwhelming and completely obscured the toxicity of the metals. Acclimation to continuous small dosages of metals offered little or no protection from large slug doses.

In the Taft Center experiments with copper, both cyanide and sulfate compounds were used with continuous dosing. Losses in efficiency up to 7 percent were noted for concentrations from 10 to 25 mg/1. The greatest loss of efficiency was found to occur with the higher concentrations of cupric ion. Acclimation resulted after 48 to 100 hours of contact. It was further noted that the sulfate ion was adsorbed by the biological mass and slowly released so as to exert a toxic effect long after the toxic substrate was removed from the system and replaced by a non-toxic substrate. Apparently the cyanide ion was degradable and posed no special problem. ($HCN \rightarrow H_2O + CO_2 + NO_3^-$)

Continuous doses of nickel in excess of 2.4 mg/1 exhibited toxic effects. The expectation that increased sulfide concentration in the influent stream would cause precipitation of nickel sulfide and a resulting decrease in toxicity was not realized.

When chromium was introduced as potassium chromate only a slight decrease in efficiency was noted even for concentrations of chromate ion as high as 400 mg/1. At 500 mg/1 chromate ion concentration increased turbidity and suspended solids were noted with the effect being evident up to 24 hours after the slug passed through the system.

Increased ammonium ion concentration was noted in the effluent in all cases. Toxicity is evidenced by the inhibition of the nitrifying process. The decrease in nitrification due to the presence of chromate ion was observed for as long as 10 days after a slug had passed through the activated sludge unit. This effect occurred for the smallest concentrations of chromate ion tested.

Ingols and Fetner report extreme bulking and accumulation of filamentous growth due to chromate ions in the substrate of an experimental

aerobic waste treatment unit. (14) They also report an increase in toxicity with decreasing nutrient strength. Reid, et al. in their studies using continuous feeding and toxic dosing of attached slimes make the same observation. (8) Toxicity in a substrate containing a fixed food concentration was much less than was found in batch studies where food concentration constantly decreases.

Kugelman and McCarty report their findings that toxicity increases with increasing valence and increasing atomic weight of dissolved metal. (15) They also discuss the cause of the toxic effect of cations. One theory advanced states that toxicity results when an enzyme necessary for metabolism unites with a metallic ion. Stimulation occurs when the metallic ion acts as an enzyme activator; when all of this enzyme present is activated the metal then unites with some other enzyme. The removal of this second enzyme from the metabolic pathway causes inhibition of growth or reproduction or both. Perhaps acclimation is the result of increased production of an adaptive enzyme which reacts with the previously toxic metallic ion. Although the study was restricted to the anaerobic process, the observations seem relevant.

Heukelekian and Gellman used the Warburg Respirometer to measure the toxic effects of certain metal ions on biochemical oxidation. (16) Various doses of metals were introduced into filtered sewage samples and the oxygen utilization measured over a period of days. Results were reported as the cumulative percent oxygen used as compared with the oxygen used in 5 days by the control sample. Extensive comparisons are difficult because the only concentration of ions common to all the metals studied is 10 mg/1. Table 2 and Figure 2 show the results for the control sample and the metals copper, hexavalent chromium and nickel at a concentration

of 10 mg/1. The graphs in Figure 2 indicate the early shock effect due to both nickel and copper. There was so little difference between the graphs of these two metals that they overlap and have been plotted as one line. The chromate ion shows little toxic effect.

TABLE 2

CUMULATIVE PERCENT OXYGEN USED BY VARIOUS SEWAGE SAMPLES CONTAINING METALLIC IONS

Time (days)	Control	Copper 10 mg/1	Chromate 10 mg/1	Nickel 10 mg/1
0.75	47	. .	40	. .
1	58	3	49	8
1.25	64	10	. .	11
2	81	51	68	48
3	91	61	81	61
4	96	64	90	65
5	100	66	97	68

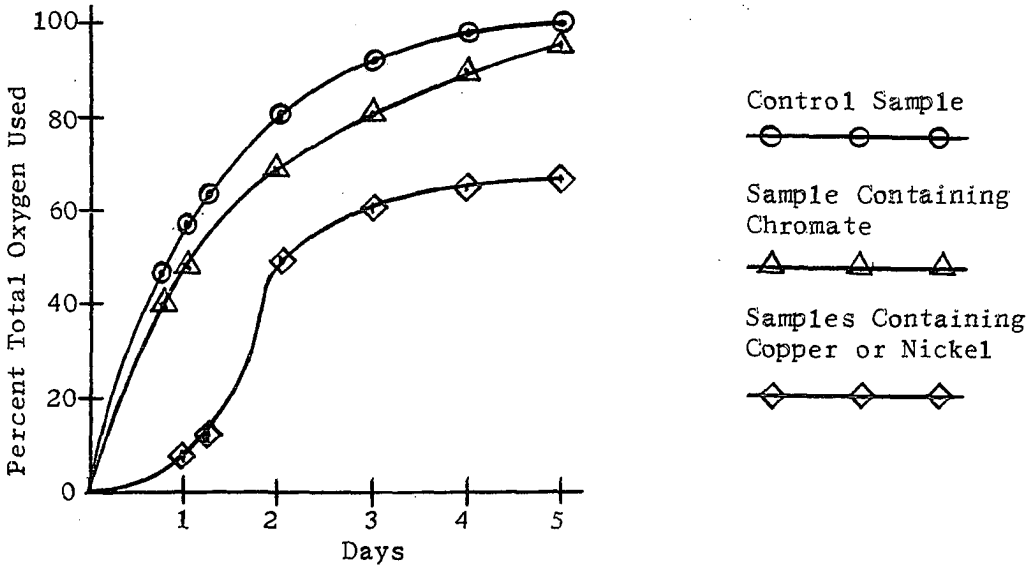


Figure 2.--Oxygen Uptake by Sewage Samples Containing Copper, Chromate, and Nickel Ions

Table 3 gives the percent oxygen used by various concentrations of copper, chromate, and nickel in 5 days. Figure 3 is a graphical representation of the data. A plateau is noted between concentrations of

TABLE 3

PERCENT OXYGEN UPTAKE IN FIVE DAYS BY SEWAGE SAMPLES CONTAINING METALLIC IONS

Concentration (mg/1)	Copper	Chromate	Nickel
0	100	100	100
1	78	. .	. .
2.5	72	. .	. .
5	68	. .	. .
10	66	97	68
25	66	90	8
50	5	85	5

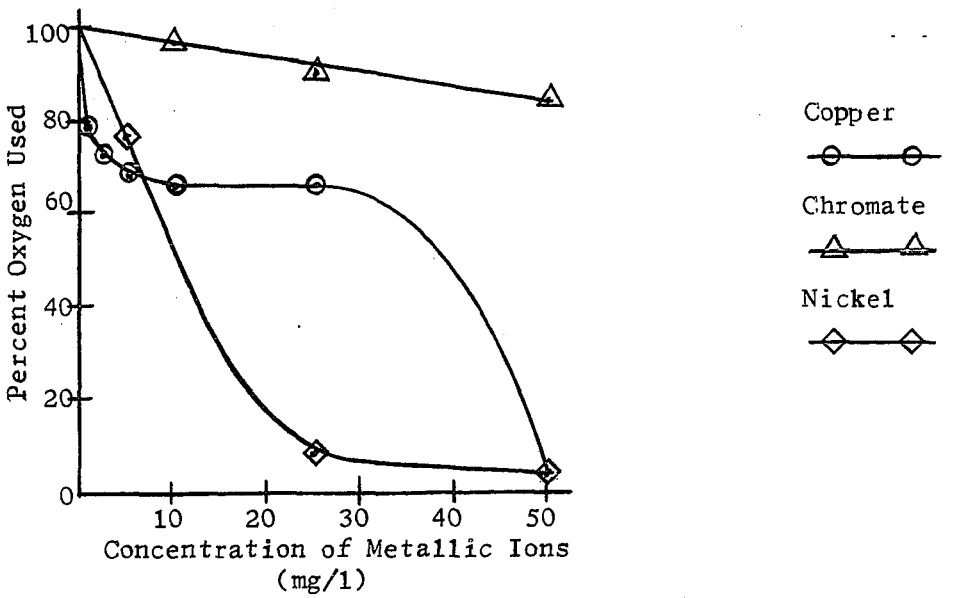


Figure 3.--Oxygen Uptake by Sewage Samples Containing Various Metallic Ion Concentrations

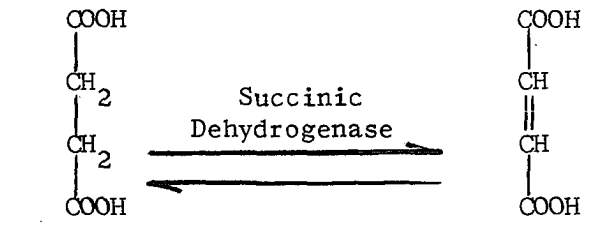
5 and 30 mg/l of copper. The shape of this curve is similar to that in Figure 1, page 6. Chromate toxicity appears to increase linearly, nickel toxicity increases rapidly and then levels off. Possibly some resistant species survive at high nickel concentrations but do not multiply. The data obtained by Heukelekian for copper and nickel is in contradiction to that reported by Alberty (20) and to that obtained in the current study.

Although the engineering literature abounds with references to the toxicity of industrial wastes little is said about the reasons for this toxicity and a search of the journals of the "pure" sciences must be made. Many citations are noted about toxicity and growth, metabolic and enzymatic inhibition. However, much of the work is extremely specific and a considerable amount of reading and integrating is necessary to separate the superfluous from the relevant to provide an overall picture of basic concepts.

The general concensus of the authors researched is that most toxicity is due to enzyme inhibition. There are several kinds of inhibition discussed in the literature, some seem to be of concern in the current study. (17)

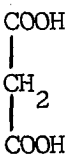
Competitive inhibition refers to a reversible reaction in which an enzyme and an inhibitor are combined. Usually the enzyme is capable of activating several related compounds, only one of which is specific for the desired reaction. A necessary characteristic of competitive inhibition is the decrease in inhibitory effect with an increase in substrate concentration. Competitive inhibition was noted by Reid. (8) He found evidence that excess food reduced the toxic effect of heavy metallic ions. The action of succinic dehydrogenase on succinic acid to form

fumaric acid is competitively inhibited by malonic acid. The reaction is indicated by:



Succinic Acid Fumaric Acid

Malonic acid has a structure sufficiently similar to succinic acid to "tie up" the dehydrogenase:



Non-competitive inhibition occurs when an inhibitor combines with a non-reactive site on either an enzyme or substrate molecule. It is generally a non-reversible reaction; substrate concentration has no effect on the amount of inhibition. The inhibitor may also combine with the enzyme substrate complex and lead to decreased dissociation of the complex. Iodoacetic acid combines with a sulfhydroyl group to inhibit the action of triose phosphate dehydrogenase in the Embden-Meyerhoff pathway of glucose metabolism.

Irreversible protein-inhibitor reactions can also occur. These reactions may cause protein denaturation, hydrolysis or oxidation, effectively removing the protein and its associated enzyme from the metabolic reaction. Quaternary ammonium compounds, such as cetylpyridinium chloride, are examples of inhibitors which utilize this mode of action. Silver forms colloidal compounds with proteins in the cell to produce bacteriostasis.

Sizer (18) discusses enzyme inhibition by metals and defines and illustrates competitive and non-competitive inhibition by the use of a graph, reproduced in Figure 4. The three curves succinctly illustrate

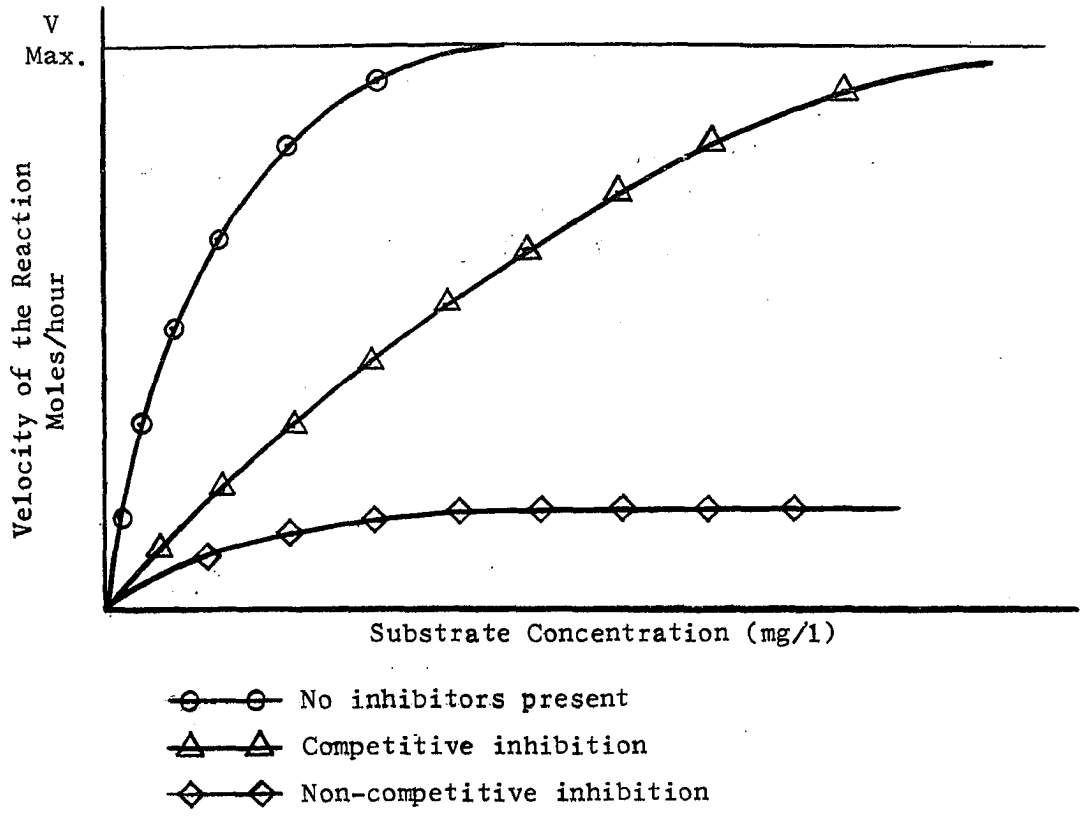


Figure 4.--Effects of Substrate Concentration on Inhibition

the definitions given in the previous paragraphs. The competitively inhibited reaction eventually reaches the maximum velocity; the reaction which is non-competitively inhibited never reaches this velocity.

According to Sizer, there are two modes of enzyme inhibition by metals, formation of metalloenzymes and the formation of metal enzyme complexes. Metalloenzymes are firmly bound metal-protein molecules with the metal being found at the active protein sites. Proteins with high affinity for metals often form these complexes. The inhibition of oxygen

transport when carbon monoxide attaches itself to metalloporphyrin in the hemoglobin is an example.

More important in the present study is the formation of loosely bound metal-enzyme complexes which often completely remove the reactants from the system, usually by dialysis or chelation. Fluoride inhibition of the magnesium activation of enolase and the sodium replacement of potassium in glycolysis are given as examples.

Melroy and Spencer studied the effects of unfavorable environments on unicellular species and concluded that the adaptive enzymes made it possible for the survival of a strain of microorganisms for short periods of time, but continuous exposure would eventually destroy the species. Intermittent exposure and recovery periods prolonged the life of the strain but did not produce immortality. The cultures died when later exposed continuously to the unfavorable environment. (19) Reid reports similar findings. (8)

Albert in an early study which preceded his book Selective Toxicity suggests that amino-acids form stable compounds with various heavy metals. The order of stability is given as:

$$Cu^{++} > Ni^{++} > Zn^{++} > Co^{++} > Cd^{++} > Fe^{++} > Mn^{++} > Mg^{++}$$

Competition in solutions containing mixtures of the metallic ions is proportional to the relative stability. (20) This conforms to the observations obtained in this investigation.

In summary, most of the previous investigations of the toxic effect of metallic ions on the biological treatment of industrial waste waters have studied the activated sludge process or the anaerobic digestion process. In Reid's studies (4, 8) of attached slimes, slug doses were investigated for low concentrations of ions, up to 3 mg/l. The study of

continuous dosing used higher concentrations. No reports of studies of mixtures of metallic ions have been found in the literature. Some studies of the toxic effects of organic complexes and anions have been made.

Many variations and combinations of the basic biological wastewater treatment processes have been studied; this gives the impression that much of the work is repetitious. Closer examination of the literature shows this to be a false impression. In spite of these variations in treatment processes the results reported have been complimentary; some deviation is to be expected when working with a dynamic microbial population.

With these ideas in mind and with a specific application, namely the degradation of plating wastes, this author attempts to define and describe the toxic effects of slug doses of divalent copper, hexavalent chromium and nickel ions on attached sewage slimes. Concentrations up to 10 mg/l will be used; the metallic ions will be added to a nutrient substrate singly and in pairs. The effects of substrate concentration in the absence of toxic metallic ions will also be studied.

In order to restrict the number of variables only one nutrient substrate will be used, lactose. In order to better simulate the conditions found in a conventional treatment plant receiving unexpected slug doses of toxic material, pH will be monitored but not controlled. The author realizes that toxicity is a function of pH and nutrient concentration, but assumes that pH changes during each experiment will be approximately the same from experiment to experiment.

CHAPTER II

EXPERIMENTAL METHODS

Reid (22) experimented with the application of radioisotope tracer techniques to waste-water treatment at the Oak Ridge National Laboratory and developed the basic non-electronic equipment used in the investigation leading to this report. As indicated in Figure 5, the equipment consists of a series of glass drums, frosted on the outside by sandblasting, mounted on pulleys and turned by a central shaft driven by a geared down electric motor. The glass drums rotate partially submerged in pans containing a nutrient broth. In this way the organisms growing on the drums are continuously exposed to both a substrate and oxygen, e.g., slime, water, air interface. The growth of attached slimes was started by initially "seeding" the nutrient broth with raw sewage obtained at the local sewage disposal plant. The pans containing the nutrient broth were emptied and refilled with a new broth quite frequently until a thick slime was obtained. Changing the nutrient broth often assured an ample supply of food for the slimes and also served to remove wastes and metabolic by-products.

The slimes grown in this manner consist of a mixture of aerobic microorganisms. Considerable "tinkering" was necessary to learn to grow a thick slime which would remain attached to the drum. Inner layers of slime, covered by subsequent growth, were unable to get the necessary food

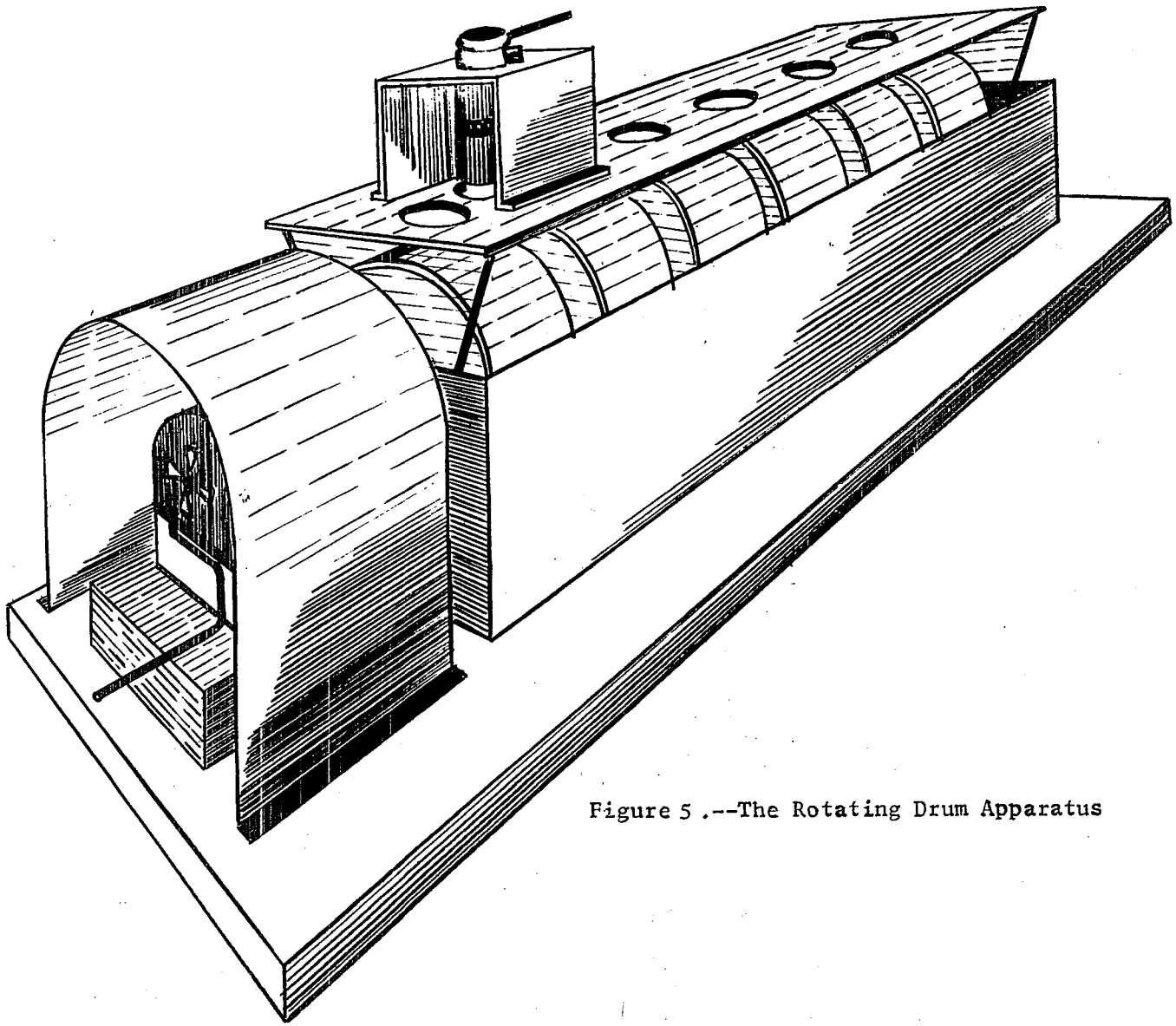


Figure 5 .--The Rotating Drum Apparatus

or oxygen to carry on metabolism. The true aerobes probably died and the facultative aerobes became predominant. With the food supply severely limited, endogenous respiration produced gases; these gases so reduced the tractive forces between layers of slimes and between the slimes and the drums that sloughing occurred. It was learned that sloughing could be prevented by severely limiting the available food as soon as a satisfactory growth was obtained. In this way a matrix was built on which new slime layers could be quickly grown. This matrix enabled the author to keep an experiment on stand-by readiness; a new slime growth could be developed in less than 24 hours.

Cell growth depends on the ability of cells to utilize the available substrate. The conversion of available substrate to energy in a form which can be used by the cell is called respiration. Early microbiologists (23) defined respiration as occurring only when oxygen was absorbed, combined with food and the oxidized product excreted. Pasteur (21) in his studies of anaerobic microorganisms directed attention to a broader viewpoint in which respiration is an exothermic reaction yielding free energy to living organisms. A more recent definition states that respiration is any energy-yielding oxidation in which the oxidant is an organic compound. (24)

Turney (25) states that it is not possible to give a precise definition of oxidation but suggests three classes of reactions be considered:

1. Loss of electrons
2. Loss of hydrogen
3. Acquisition of oxygen.

The reversible reaction:

$$Fe^{++} \underset{\text{reduction}}{\overset{\text{oxidation}}{\rightleftharpoons}} Fe^{+++} + e^-$$

illustrates the first class of reaction. Here ferrous iron is oxidized to ferric iron through the loss of an electron. The reverse reaction reduces ferric iron to ferrous iron when Fe^{++} gains an electron.

The second class of reaction, the loss of hydrogen, is called dehydrogenation. In this case hydrogen is transferred to some "hydrogen acceptor". For example, ethyl alcohol is oxidized, forming acetaldehyde, when two hydrogen atoms are lost.

$$CH_3CH_2OH \longrightarrow CH_3CHO + 2H^+ + 2e^-$$

The hydrogen lost in the above reaction and in similar reactions is "accepted" or gained by another suitable substance, often an enzyme. The enzymes in turn are dehydrogenated or re-oxidized in another process and thus readied for use again.

In many cases oxygen combines with the hydrogen lost by the enzymes.

$$H_2 + \tfrac{1}{2}O_2 \longrightarrow H_2O$$

This illustrates the third class of oxidizing reactions.

There is a great diversity of substrates available to the microorganisms found in a biological wastewater treatment system. The oxidation of the substrate during respiration yields the energy necessary for the life of the organisms. The energy obtained from oxidation is stored in the chemical bonds of several phosphate compounds. In the Kreb's cycle of metabolism pyruvic acid is degraded to carbon dioxide and water; for every mole of pyruvic acid degraded, 273 kilocalories of energy are released. Phosphate compounds are capable of storing about

44 percent of this energy. The rest is lost as heat. In summary, it can be seen that phosphorous plays an important role in metabolism.

Because of the cellular need for phosphorous, Reid used P-32 to trace metabolism. Biological waste treatment processes are usually monitored and studied by measuring the uptake of oxygen which we have seen can be correlated to the respiration of the microorganisms involved. Many techniques are available to measure oxygen uptake. The tracer technique has been shown to be capable of representing the oxygen uptake. (26) Thus, bioactivity or uptake of nutrients is measured in terms of either metabolism or oxygen uptake. This uptake over a period of time can be formulated in many ways. The one used by most researchers and by Reid was a first order differential equation where

$$\frac{dy}{dt} = -k' (L-y) \qquad (1)$$

$\frac{dy}{dt}$ is the rate of substrate utilization,

k is a proportionality factor often called the "reaction rate constant", and

L-y is the substrate remaining.

The solution of this equation yields

$$\text{Ln } (L-y) = k't \qquad (2)$$

from which we obtain

$$\text{Log}_{10}(L-y) = -2.303\ k'y \qquad (3)$$

or $$\text{Log}_{10}(L-y) = -K't \qquad (4)$$

where $K' = 2.303\ k'$.

Rewriting equation (4) in non-logrithmic form and dividing by L gives:

$$\frac{L-y}{L} = \frac{10^{-K't}}{L} = 10^{-K''t} \qquad (5)$$

here $\frac{1}{L} = 10^{-x}$ and $10^{-K''t} = 10^{-K't-x}$

Finally, multiplying equation (5) by 100 gives an equation expressing the percent remaining substrate in terms of "K", the reaction rate constant, and time.

$$\frac{L-y}{L} \times 100 = 10^{-K''t} \times 100 \qquad (6)$$

$$\frac{L-y}{L} \times 100 = 10^{-Kt+2} \qquad (7)$$

When equation (7) is plotted on semi-logrithmic graph paper a straight line with a slope equal to "K" is obtained.

$$\log\left[\frac{L-y}{L} \times 100\right] + Kt-2 = 0 \qquad (8)$$

The reaction just described is called a first order reaction and a plot of this reaction yields the straight line just discussed. Unfortunately the entire reaction is not a pure first order reaction but rather a mixed reaction probably of several different orders. Before "K" can be obtained the "straight line" portion of the reaction must be isolated. This can be done graphically. (27)

Figure 6(a) is a typical plot of the substrate used by a microbial population as a function of time. Figure 6(b) is a plot of the food remaining as a function of time. These figures help to define "L", "Y", and "L-y". L is the total substrate used, also called the "ultimate substrate", y is the substrate used in any time interval. L-y is the difference between the total substrate ultimately used and the substrate used in time, t. It is, then the substrate remaining. In the present study L and y refer to the P-32 in the substrate. L is the total amount of P-32 used by the attached slimes and y is the P-32 used in time, t.

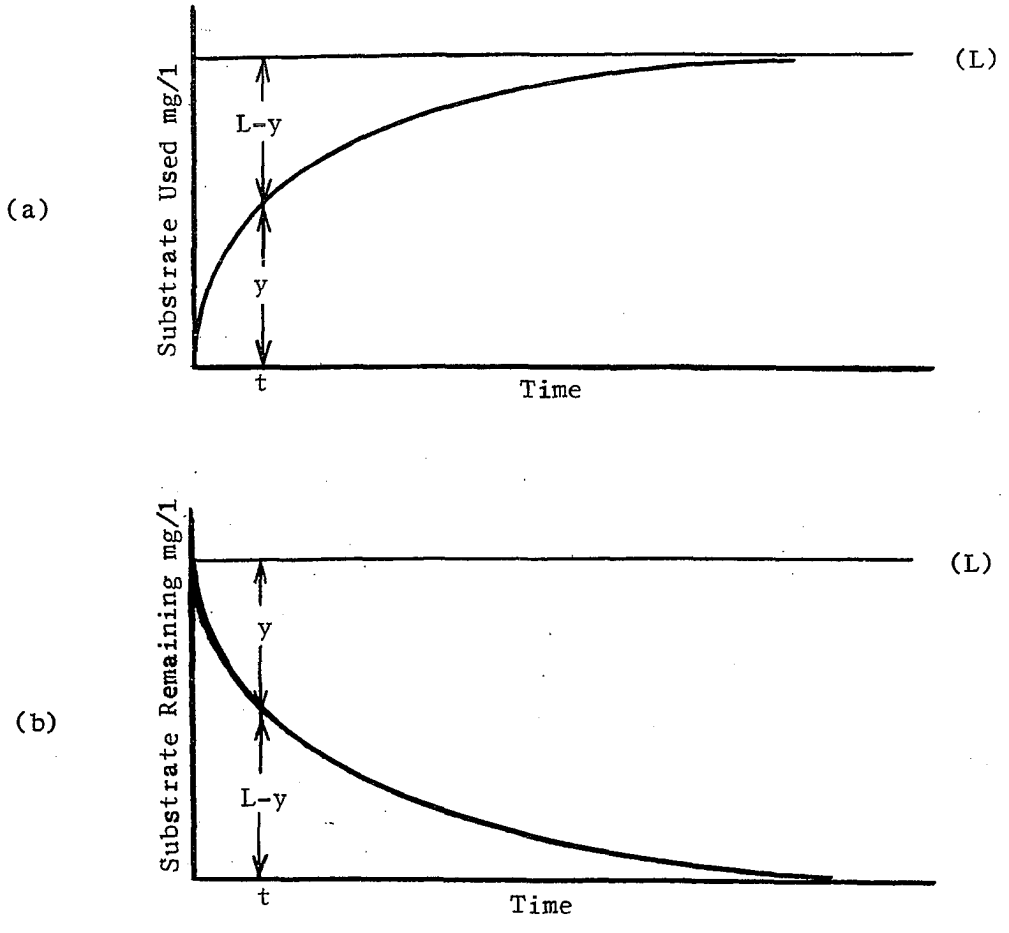


Figure 6.--Graphical Definitions of L, y, L-y, and t

Phosphorous-32, P-32 is a radioactive isotope of phosphorous with a half life of approximately 14 days. It decays with the emission of a nuclear electron, a beta particle, to form sulfur 32, a non-radioactive isotope of sulfur. The emitted beta particle has a maximum energy of about 1.7 Mev. This radiation particle readily reacts with standard nuclear detection equipment.

When P-32 is added to the substrate in which glass drums and the attached slimes are rotating the P-32 is removed from the substrate by the microorganisms forming the slimes. A detector placed over the slimes

measures the uptake of the P-32 by the slimes. The rate of uptake increases quite rapidly at first and continues for about 15 minutes. During this time tests have shown that the uptake is almost entirely due to adsorbtion. (26) Subsequent uptake is a combination of adsorbtion and assimilation. It is suspected that adsorbtion is a necessary physical process preceding assimilation. The increase in phosphate uptake rate continues until growth stops. After cessation of growth a slight loss of phosphate was noted. This is probably due to sloughing or licing of cells. Data from a typical experiment and the graph of the data is shown in Table 4 and Figure 7.

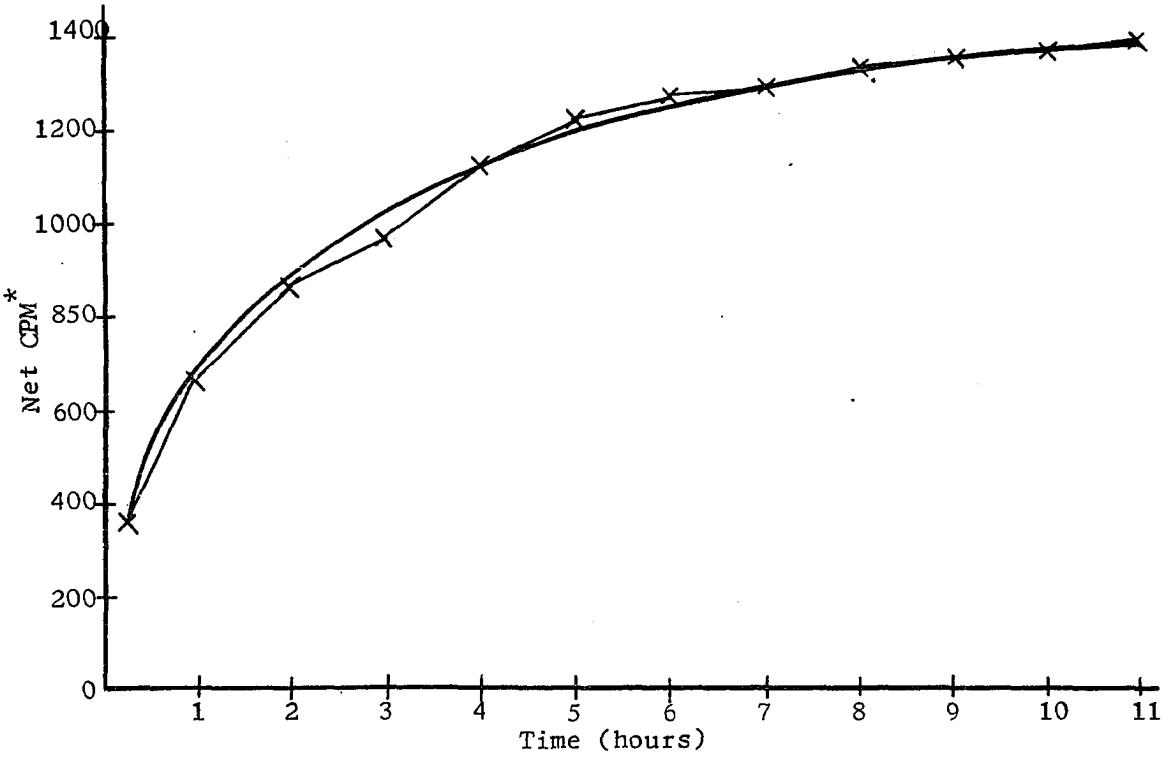
Each count per minute as obtained from the G.M. tube and scalar represents the detection of a nuclear decay of beta particle. Many beta particles go undetected because they do not pass thru the G.M. tube. Shielding around the G.M. tube permits only those beta particles which come from the slimes to reach the tube. Therefore, detection of a beta particle means the slimes had removed an atom of phosphate from the substrate.

In order to obtain a continuous record and to "smooth out" the data already obtained, a recording rate-meter was employed as an auxillary detection device in some of the experiments. The recording rate-meter receives electrical impulses from the G.M. tube in the same manner as the scalar, but an integrating circuit adds and averages the incoming pulses over a short period of time and records the results as counts per minute on a moving chart. Correlation between the two detection methods was quite good. Figure 8 is a representation of the chart produced by the recording rate-meter.

TABLE 4

TYPICAL DATA OBTAINED IN THE EXPERIMENT

Time	CPM
0.0	384.0
1.0	660.0
2.0	854.0
3.0	969.0
4.0	1119.0
5.0	1217.0
6.0	1275.0
7.0	1285.0
8.0	1333.0
9.0	1354.0
10.0	1376.0
11.0	1396.0



*The original data, total counts per minute, has been changed to net counts per minute by substracting the background. Net counts/minute = Total counts/minute - background counts/minute.

Figure 7.--Typical Curve of Data (Table 4) Obtained in the Experiment

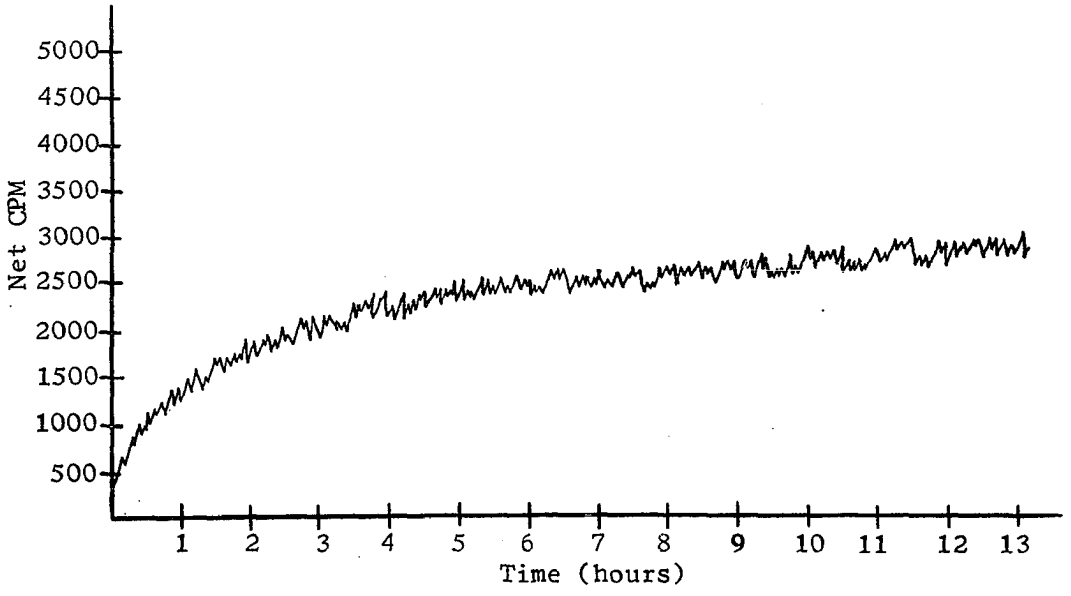


Figure 8.--Reproduction of Typical "Recording Rate Meter" Chart

The experimental data can be treated to yield "K", the reaction coefficients. The "K" values obtained under different conditions can then be compared. Both Figures 7 and 8 indicate a maximum number of counts per minute (maximum count rate). Letting the maximum count rate equal L and the hourly count rate equal y, it is possible to compute the percent remaining phosphorous, $\frac{L-y}{L} \times 100$. When corresponding values of the logarithm of $\frac{L-y}{L} \times 100$ and time are plotted on quadrile paper a graph such as Figure 9 is obtained. The use of standard linear regression equations and techniques for the linear part of the curve so obtained yields "K", the slope of the line. It should be pointed out that Figure 7 and Figure 9 are graphs of the same data treated in different ways.

The methods used to find "K" were developed and justified by Reid and Varma. (26) A mechanism using rotating glass drums, similar to the equipment used in the present study was placed in a sealed plastic box

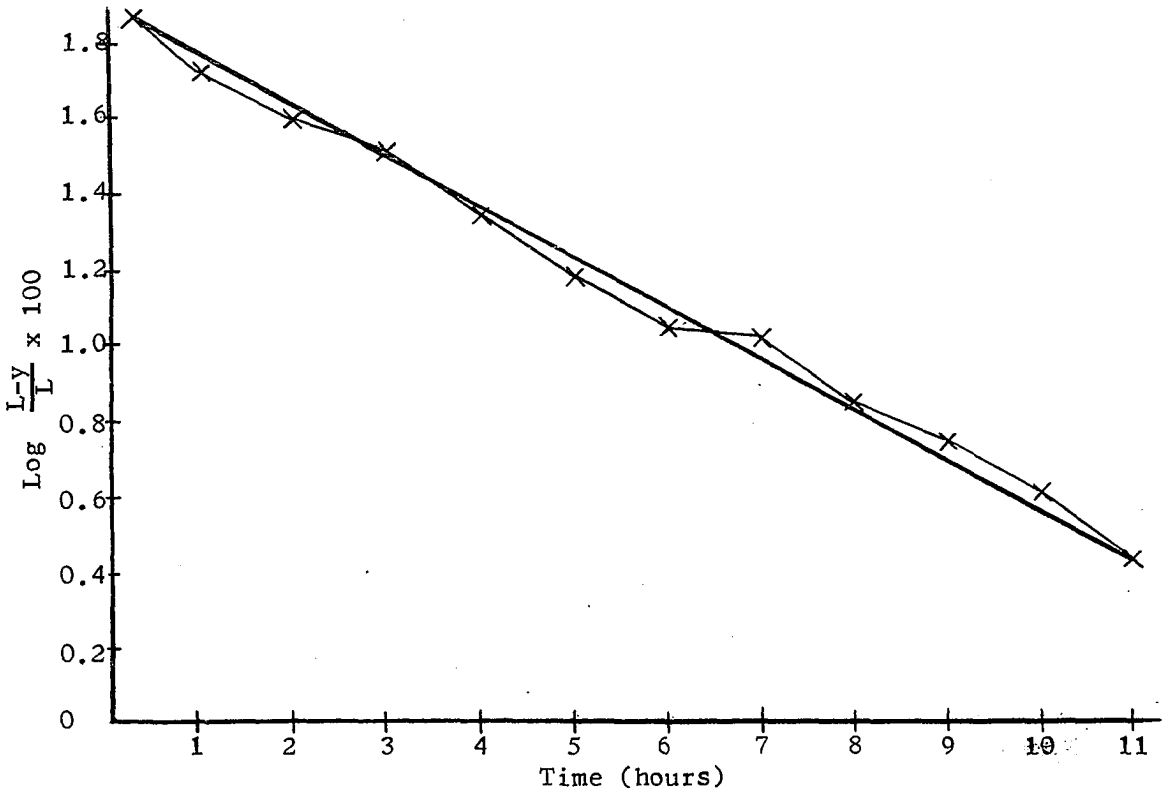


Figure 9.--Plot of Log $\frac{L-y}{L}$ x 100 vs Time, Using Data in Table 5.

TABLE 5

DATA USED TO PLOT FIGURE 9
(FROM TABLE 4)

Time	CPM	$\frac{L-y}{L}$ x 100	Log $\frac{L-y}{L}$ x 100
0	384.0	73.2	1.864663
1	660.0	53.9	1.732213
2	854.0	40.4	1.606898
3	969.0	32.4	1.510922
4	1119.0	21.9	1.341778
5	1217.0	15.1	1.179925
6	1275.0	11.0	1.044861
7	1285.0	10.3	1.016649
8	1333.0	7.0	0.847782
9	1354.0	5.5	0.746550
10	1376.0	4.0	0.606886
11	1396.0	2.6	0.423239

with a pure oxygen atmosphere. A G.M. tube was also sealed in the box so P-32 uptake could be measured. Carbon dioxide evolved by respiration was removed by an appropriate absorber. Manometric pressure decreases in the oxygen atmosphere represented oxygen utilization by the microorganisms and was compared with simultaneous P-32 uptake. They concluded that P-32 uptake was correlated to oxygen uptake and the growth rate constants, "K", obtained by either method were equally valid. P-32 uptake data was reproducible. It was observed that the pH of the nutrient broth decreased during the experiment probably due to metabolic by-products. Reid and Varma (26) further concluded that the attached slimes consisted of a mixed population. No attempt to identify individual species was reported.

Specific Experimental Techniques

Preliminary studies, during the present investigation, were conducted to refine techniques and to confirm adequate reproducibility. "K" was reproducible when similar nutrient concentrations were used; pH values were found to vary during an experiment. Initial pH values averaged 8.3; the final pH of the substrate was considerably lower but never less than 6.0. The initial pH of 8.3 and stabilized pH of 6.8 was a reproducible steady state condition. The microbial populations of the attached slimes are apparently prodigious acid producers; attempts to stabilize the pH of the substrate at 7.0 required excessive amounts of phosphate buffer solutions. The use of large amounts of phosphate in the buffer compounds created greater problems than utilizing a consistent and reproducible phenomenon, a phenomenon that was believed more representative of real life conditions. Isotopic dilution was suspected

as the cause of these problems. Isotopic dilution results when the ratio of non-radioactive phosphorous to P-32 is high; although the attached slimes assimilate phosphorous, the increase in phosphorous uptake is undetectable because of low specific activity. Several acetate buffers were also tried; buffering capacity was extremely limited. When acetate buffers were used, the uptake rates of P-32 were very erratic and it was impossible to reproduce "K" values; possibly because the acetate was used as a food source supplementary to the lactose.

The experimental broth was always "spiked" with 3 microcuries of P-32. Larger amounts of P-32 tended to overload the detection apparatus, smaller amounts introduced statistical problems. P-32 was obtained from a supplier in a carrier free state, meaning the only phosphorous present was P-32. At the beginning of the investigation it was feared that the substrate might be phosphorous limiting, that there was insufficient phosphorous to permit metabolism of all the lactose present. Aliquots of the substrate were withdrawn both before and after an experimental run and evaporated to dryness under a heat lamp. The maximum decrease in radioactivity was about 35 percent. Apparently sufficient phosphorous remains for metabolism.

The nutrient broth was contained in three 3/4 x 5 1/2 x 9 1/2 inch aluminum bread pans. Each pan was large enough that two glass drums could be rotated side by side in the same pan if so desired. The drums were about 3 inches apart. It was the hope of the investigator that the attached slimes cultured in the same pan would have similar growth characteristics. The pans containing the experimental broths were sometimes arranged in a similar manner so slimes grown together in a common nutrient broth were placed in a common broth for an experimental run. When

this was done the uptake rate of P-32 by the attached slimes on one drum closely approximated the uptake rate of P-32 on the other drum. Other experiments were conducted in which drums previously sharing a common broth during the culture period were placed in separate pans containing similar broths for the experiment. Again, uptake rates were comparable. (see page 50.)

Varma (26) showed that P-32 uptake was not a function of slime thickness but only of the slime surface. In order to ensure that comparable slime surfaces were used for each experiment the nutrient broth was replaced with a broth nearly identical to the experimental broth about 15 hours before an experimental run. The replacement broth contained no toxic metallic ions and no P-32; the P-32 was replaced with potassium monobasic phosphate. The P-32 was obtained from a commercial supplier as potassium monobasic phosphate also.

Lactose concentrations were determined by evaporating an aliquot of previously prepared stock solution to dryness and weighing. Ammonium, chromate, and copper concentrations were found using colorimetric methods outlined by the Hach Chemical Company, Ames, Iowa. The Bausch and Lomb Spectronic 20 colorimeter was used for these measurements. Gravimetric methods were used to determine nickel content; the dimethylglyoxime complex of nickel was used to precipitate the nickel for this measurement.

The uptake of P-32 was measured with a Radiation Counters Laboratories Scalar Rate Meter Model 20324. A halogen quenched, end window G.M. tube was used; the window thickness was 4.5 mg/cm^2. The recording rate meter was manufactured by Atomic Accessories, Inc.

The substrates were made up using several combinations of nutrient and toxic materials. In all cases 5 mg/l of ammonium as ammonium sulfate

was used. In order to determine the effect of nutrient concentration, four different lactose concentrations were used, 250 mg/l, 500 mg/l, 750 mg/l and 1000 mg/l. When preliminary studies indicated that 1000 mg/l of lactose produced the largest growth rates the decision was made to use this concentration in all experiments involving toxic metallic ions.

The concentration of toxic metallic ions was varied in increments of 2 mg/l from zero mg/l to 10 mg/l. Mixtures of metallic ions were also used. In several cases experimental combinations of toxic metallic ions were used in the substrate but the absence of P-32 uptake indicated that growth was completely inhibited and the experiment was abandoned. In spite of efforts to control sloughing there were times when sloughing occurred during an experimental run. When this happened the data obtained was discarded.

The laboratory was air conditioned and kept at a constant temperature of 24°C. Rather than trying to build elaborate constant temperature baths all experiments were run at room temperature.

CHAPTER III

RESULTS AND CONCLUSIONS

The discussion on page 21 emphasizes that the first order reaction must be separated from the others. To do this the original data was converted to $\log \frac{L-y}{L} \times 100$ and plotted against time. A first order reaction or equation when thus plotted yields a straight line. Points at either end of the graph which obviously did not lie on the straight line were discarded. Visual examination was used for this step. "K" is the slope of the best fitting line after elimination of the outlying end points. All computations and plotting was done with the IBM 360/40 and IBM 1130 computers and associated equipment. Standard least square regression techniques were used to find "K" from $\frac{L-y}{L} \times 100$ vs t.

A "K" value obtained from the experimental data is quite meaningless by itself and the "K" values must be compared at various metallic ion concentrations to discern trends. The average "K" values obtained from the various blank runs have been arranged according to concentration in Table 6. A blank run is defined as an experiment in which no toxic metallic ions were added to the substrate.

A blank run was always performed concurrently with an experiment using toxic metallic ions. Lactose concentrations for the toxic and blank substrates were the same and the visible characteristics of the slimes were similar. Results for the various runs in which toxic metallic ions were used are given in Table 7. In order to make comparisons

TABLE 6

AVERAGE "K" VALUES FOR VARIOUS BLANK RUNS

Lactose Concentration (mg/1)	"K"	95 % Confidence Interval for "K"	
250	0.0985	0.0932 to 0.1038	8 runs
500	0.1234	0.1203 to 0.1265	9 runs
750	0.1580	0.1377 to 0.1783	9 runs
1000	0.2222	0.2086 to 0.2358	7 runs

TABLE 7

SYNOPSIS OF TOXIC EFFECTS OBTAINED WITH THE USE OF METALLIC IONS IN THE SUBSTRATE

	Concentration of Metallic Ion in the Substrate		Run #1 % K_o	Run #2 % K_o	Run #3 % K_o	Average % K_o
	mg/1	µe/1				
Chromate	0	0	100.00	100.00	100.00	100.00
	2	2.31	82.88	80.35	86.72	83.32
	4	4.61	64.04	73.97	71.73	69.79
	6	6.92	57.53	57.53	63.07	59.04
	8	9.22	44.86	52.05	47.72	48.21
	10	11.53	34.93	37.44	39.83	37.40
Nickel	0	0	100.00	100.00	100.00	100.00
	2	0.68	78.36	70.43	66.81	71.87
	4	1.36	52.38	61.30	. .	56.84
	6	2.05	39.83	45.22	49.12	44.72
	8	2.73	30.74	30.00	26.99	29.24
	10	3.41	23.38	20.43	20.35	21.39
Copper	0	0	100.00	100.00	. .	100.00
	2	0.63	47.34	40.30	. .	43.82
	4	1.26	41.06	38.81	. .	39.34
	6	1.89	. .	39.30	. .	39.30
	8	2.52	41.55	33.83	. .	37.69
	10	3.15	39.13	34.83	. .	36.98

easier, results have been expressed as percent of K_o; K_o is the growth rate of the attached slimes in the blank substrate. Kugelman and McCarty (15) suggest that the toxicity of metallic ions is a function of atomic weight and valence. Reid (4) makes the same observation. In order to investigate this lead further, equivalent weights have been also used as a comparison. Table 7 lists concentrations of metallic ions as both mg/l and μe/l. Complete listings of the data and associated statistical analyses are given on pages 51, 52, and 53.

Figure 10 is a plot of the percent "K_o" against concentration of toxic metallic ion on semi-logarithmic paper. This figure might be used as a predictive device. For example, Figure 10 predicts that a concentration of 7 mg/l of chromate will reduce the growth rate of attached

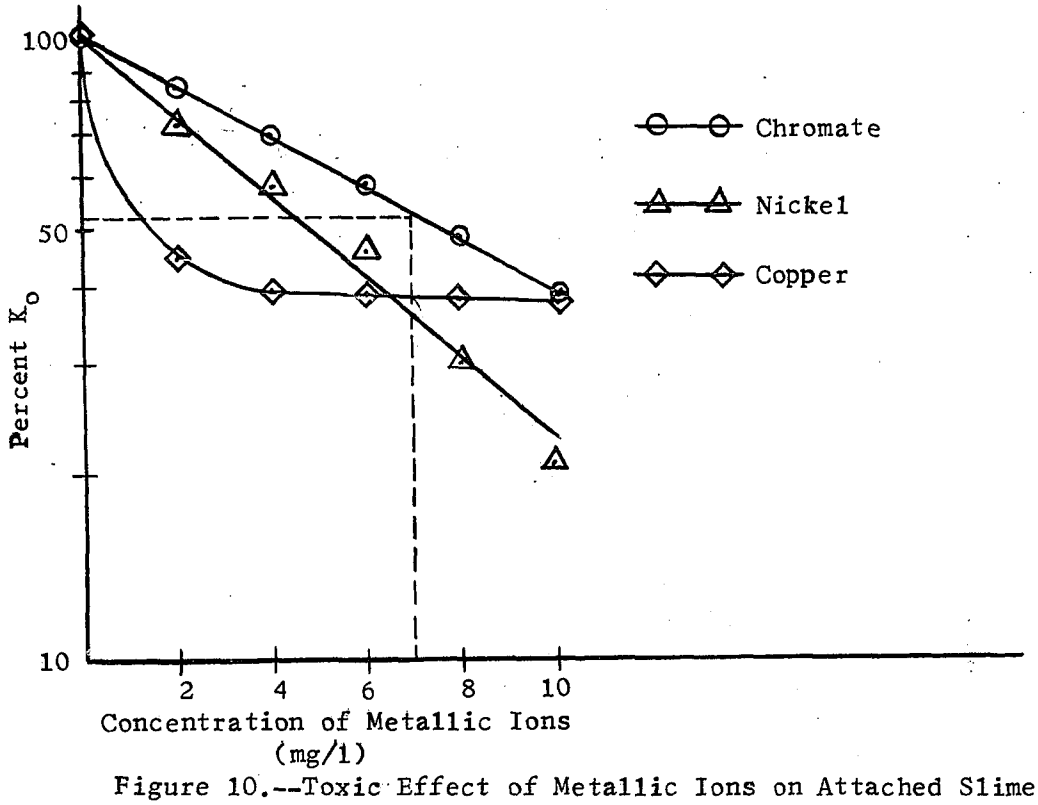


Figure 10.--Toxic Effect of Metallic Ions on Attached Slimes

slimes to 51 percent of the growth rate with no toxic metallic ions present. No attempt has been made to verify this prediction. A complete listing of the slopes and confidence intervals for the curves illustrated by the figure above is found on pages 51, 52, and 53.

Figure 11 is also a semi-logarithmic plot of the percent "K_o" against concentration of metallic ions. This time concentration is given in micro-equivalents per liter. When plotted in this fashion it is noted that the toxicity, represented by the slopes of the lines, changes. Chromate toxicity decreases slightly while the toxicity due to nickel ions shows a great increase. When percent "K_o" is plotted against mg/l of copper a very definite plateau is evident. The use of μe/l as the abscissa has the graphical effect of shortening the interval between data

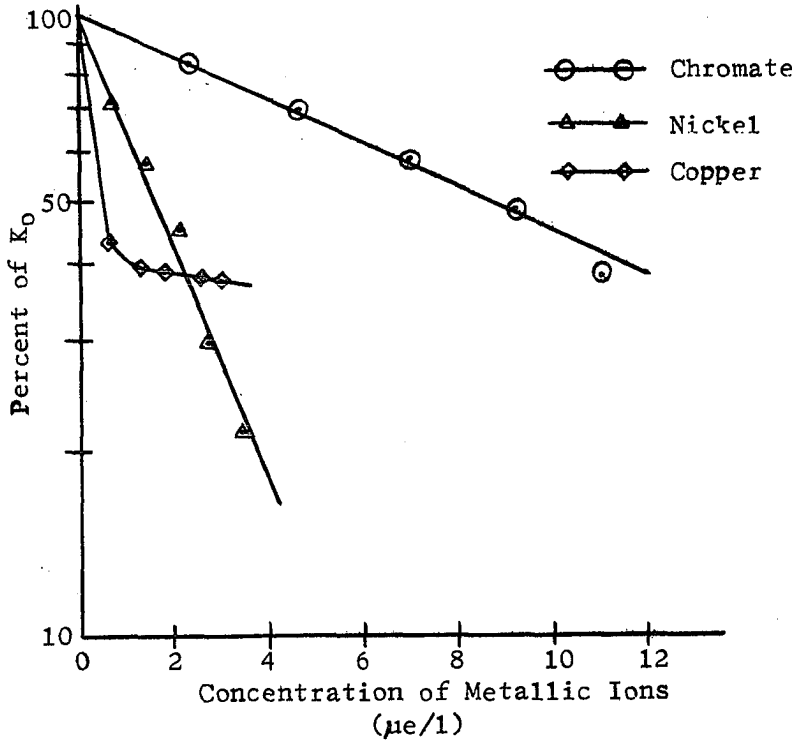


Figure 11.--Toxic Effect of Metallic Ions on Attached Slimes

points; now, the plateau disappears and it is seen that copper toxicity continues to increase.

Nickel ions in the presence of chromate ions exhibit synergistic behavior. A synergist is a substance which increases the effect of a second substance. For example, either of two drugs taken separately may produce a desirable degree of sedation; taken together the sedative effect may be greatly compounded to the point where an involuntary bodily function such as breathing is impaired and death may result.

Nickel was added to substrates containing 2 mg/1 and 4 mg/1 of chromate ions; copper was added to a substrate containing 2 mg/1 of chromate ions. Results are shown in Table 8. "K_o" is the growth rate of the attached slimes when only the chromate ions were present.

A semi-logarithmic plot of 'percent "K_o"' against concentration of metallic ions for the mixtures given in Table 8 is shown in Figure 12. Figure 13 is a similar plot; μe/1 is used instead of mg/1. Data is incomplete and sketchy, but the line representing 4 mg/1 chromate ions plus nickel ions and the line representing the mixture of 2 mg/1 chromate ions and copper ions appear to have leveled off on a plateau. Attention is drawn to the similarity with Figure 1 on page 6.

Difficulties encountered due to sloughing of the attached slimes prevented the acquisition of additional data. Where values are missing in the above tables, sloughing did occur. Subsequent attempts to repeat the experiment ended in failure for the same reason.

Table 9 lists the average "K" values as a function of mg/1 and micro-equivalents/1, μe/1, for the experimental runs using chromate and nickel ions, singly and as a mixture. Percent K_o is also listed; K_o is listed as -0.222, the mean value of "K" for all blank runs using 1000 mg/1

TABLE 8

SYNOPSIS OF TOXIC EFFECTS OBTAINED WITH THE USE OF MIXTURES OF METALLIC IONS IN THE SUBSTRATE

	Concentration of Metallic Ion in the Substrate		Run #1	Run #2	Average
	mg/1	µe/1	% K_o	% K_o	% K_o
Chromate Nickel	2	2.31	100.00	100.00	100.00
	2	0.68	57.63	61.03	59.33
	4	1.36	. .	41.31	41.31
	6	2.05	33.05	. .	33.05
	8	2.73	27.12	21.13	24.12
	10	3.41	14.41	. .	14.41
Chromate Nickel	4	4.61	100.00		
	2	0.68	. .		
	4	1.36	. .		
	6	2.05	44.62		
	8	2.73	44.09		
	10	3.41	. .		
Chromate Copper	2	2.31	100.00		
	2	0.63	. .		
	4	1.26	39.04		
	6	1.89	. .		
	8	2.52	. .		
	10	3.15	39.26		

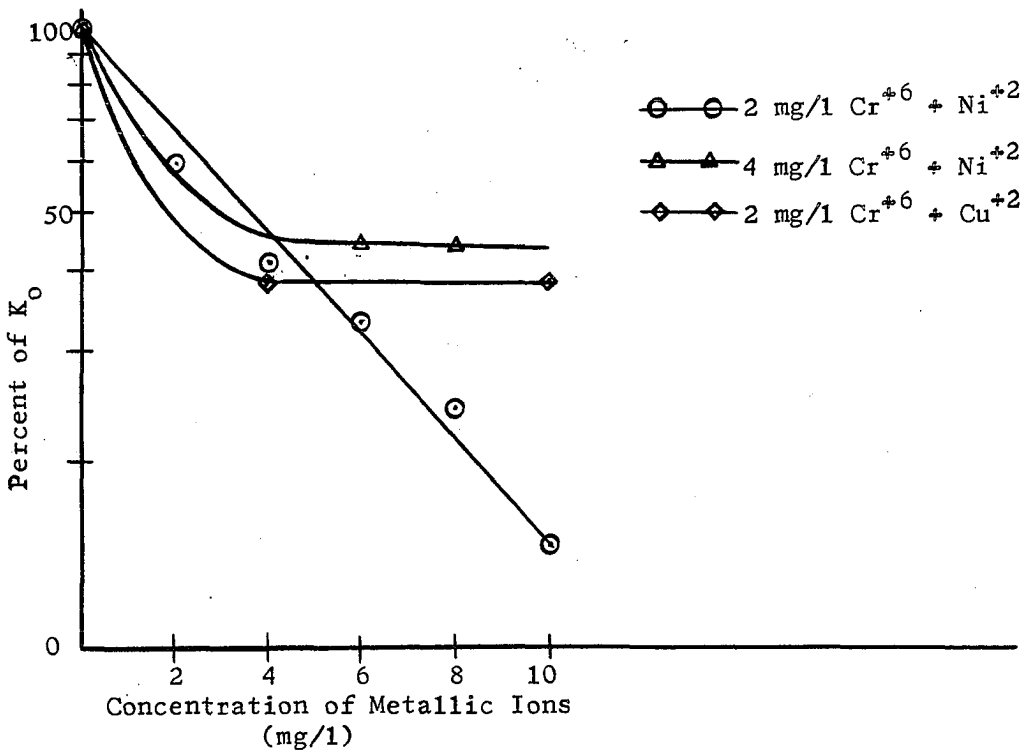


Figure 12.--Toxic Effect of Mixtures of Metallic Ions on Attached Slimes

of lactose as the substrate.

TABLE 9

TOXIC EFFECTS OF MIXTURES OF METALLIC IONS

	Concentration of Toxic Ions mg/l	μe/l	K	% K_o
Chromate	0	0	-0.251	100
	2	2.31	-0.209	83.3
	4	4.61	-0.174	69.3
	6	6.92	-0.149	59.4
	8	9.22	-0.120	47.8
	10	11.53	-0.093	37.0
Nickel	0	0	-0.229	100
	2	.68	-0.165	72.0
	4	1.36	-0.131	57.2
	6	2.05	-0.102	44.5
	8	2.73	-0.067	29.3
	10	3.41	-0.046	20.1
Mixture	0	0	-0.222	100
	4	2.99	-0.088	39.6
	6	3.56	-0.039	17.6
	8	4.35	-0.038	17.1
	10	5.03	-0.017	7.6

Figure 13 is a semi-logarithmic plot of percent K_o versus μe/l of the ions as listed in Table 9. Line PA represents percent K_o for a nutrient substrate containing chromate ions only. Line PD represents percent K_o for a nutrient substrate which contains nickel as the only toxic metallic ion. Line OB, is line PD moved from its normal position passing through K_o = 100 percent to its present position; the proper slope has been maintained in this transfer. Line PO represents the effect due to 2.31 μe/l of chromate ion. As seen in Table 9, increments of 0.682 μe/l of nickel have been added to 2.31 μe/l of chromate; line OB illustrates this. Line OC is the best fitting line for percent K_o when

a mixture of chromate and nickel is added to the nutrient substrate.

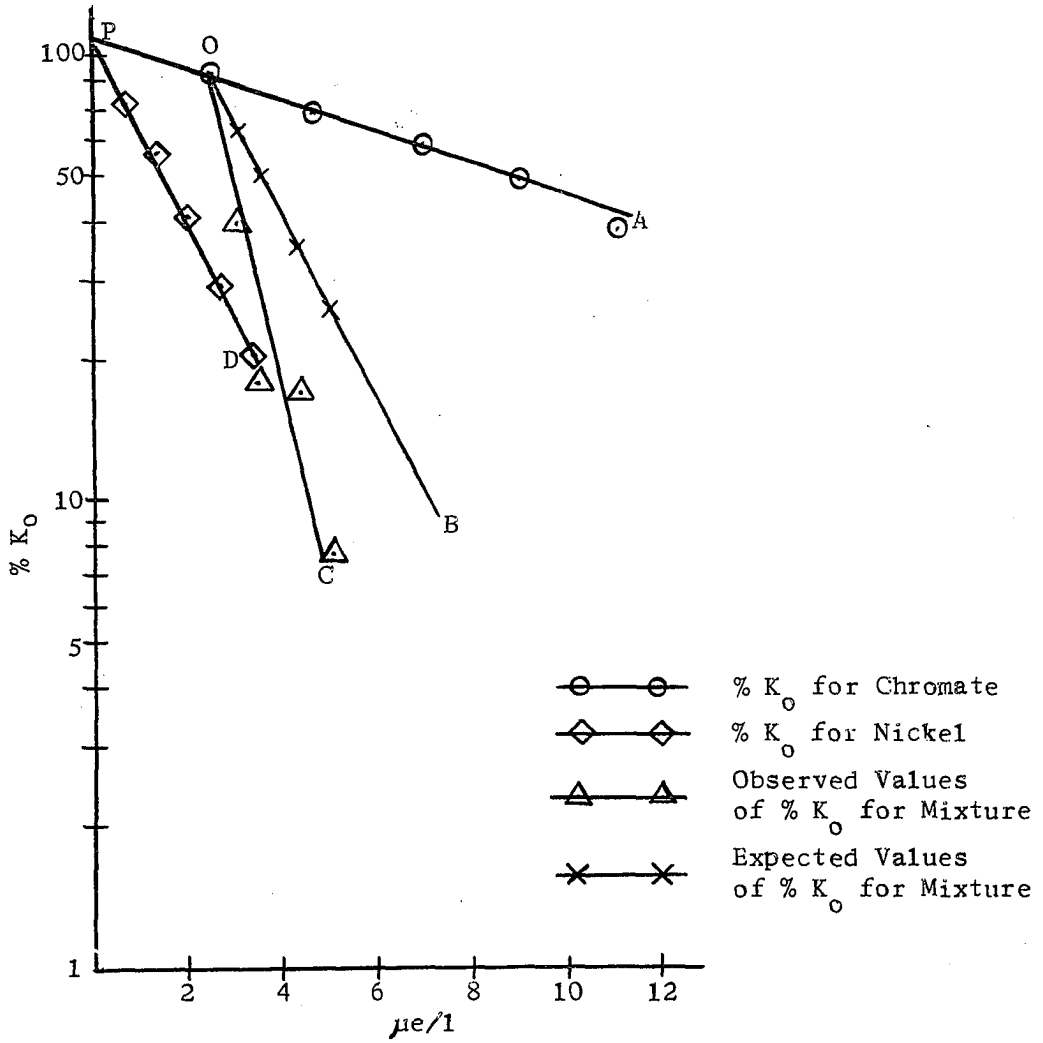


Figure 13.--The Toxic Effects of Mixtures of Metallic Ions

The literature surveyed contains no precise mathematical definition of synergism. The author proposes first a graphical definition of both synergistic and antagonistic toxic effects. If there are no synergistic effects then toxic effects should be additive and percent K_o for the nutrient substrate containing the mixture of metallic ions should be obtainable as points along line OB; the X's on line OB represent the expected values of percent K_o for the mixtures used when non-synergism

is assumed. For the case of non-synergism, line OB and line OC should coincide.

When synergistic effects are present percent K_o should be represented by a line whose slope is greater than the slope of line OB; this line should lie below line OB, as line OC does in Figure 13. The marks are the experimental values obtained when the mixture of ions was used.

Antagonistic effects are defined as those cases in which line OC lies above line OB, i.e., the slope of line OC is less than the slope of line OB. Using the definitions discussed above, nickel ions display a synergistic toxic effect on attached slimes when added to a substrate containing chromate ions.

Perhaps the percent difference in slopes between lines OB and OC can be used as a numerical measure of synergism or antagonism. More precisely,

$$\text{percent effect} = \frac{\text{Slope OB} - \text{Slope OC}}{\text{Slope OB}} \times 100$$

Negative percentage values indicate the presence of synergism, positive values represent antagonism. Several limiting cases might be discussed. When the addition of the most minute amount of a synergist stopped all activity OC would be vertical with a undefined negative slope and the synergist has an infinite effect. Of course if line OB is already a vertical line downward there will be no effect when a synergist is added. If line OB is a vertical line downward, the addition of any antagonist will cause OC to lie to the right of OB. Mathematically, the change in the slope is infinite; the biological interpretation of this phenomenon is open to question. Another case, the addition of an

antagonist might cause OC to go vertically upward; this situation is extremely unlikely and the discussion is academic.

Conclusions

In review, the objectives of this investigation were:

1. To study the toxic effects of Cr^{+6}, Ni^{+2}, and Cu^{+2} in slug doses on attached slimes.
2. To depict and explain the effects of a substrate containing known amounts of toxic metallic ions, singly and in pairs, on conventional waste treatment processes.

These objectives have been met; new questions have been brought to the foreground.

The study definitely demonstrates that slug doses of the metallic ions in question produce toxic effects on attached sewage slimes. The effect is more pronounced for some metals than for others and the severity of the effect is in all instances a direct function of the concentration of the metallic ion. Substrates containing mixtures of metallic ions exhibit a greater toxic effect than do those substrates which contain only a single species of metallic ion.

The results of the current study generally bear out and extends the conclusions drawn by previous investigators. The degree of toxicity as found in this study differs from that reported elsewhere. Variations in food concentration, type of food, microbial population differences, and experimental technique singly or in combination could well account for this observation. It is to be noted that growth rate is a function of lactose concentration, as shown in Table 6.

Figures 10 and 11 would indicate that small concentrations of copper, those less than 4 mg/l or 1.0 μe/l are extremely toxic to attached

slimes, reducing the growth rates, and as such, the efficiency of a treatment plant, to 40 percent of capacity. Further increases in copper concentration have little effect on growth rates. Perhaps toxicity increases again at much higher concentrations. Small concentrations of copper may completely decimate certain microbial species. More resistant, more slowly metabolizing species survive and continue to utilize P-32, even at higher copper concentrations.

Figures 12 and 13 indicate that similar effects may occur for combinations of nickel and 4 mg/1 or 4.61 μe/1 of chromate ions and copper with 2 mg/1 or 2.31 μe/1 of chromate ions. Lack of data makes this conclusion precarious. Combinations of metallic ions may produce reactions which reduce the tractive forces between the attached slimes and the glass drums, immediate sloughing results.

The growth rates of attached slimes in contact with substrates containing toxic metallic ions may be expressed as the percent of some "ultimate growth rate", such as "K_o". When expressed in this way there appears to be a linear logarithmic relationship between the growth rate and the toxic metallic ion concentration.

There were times when data was being collected from as many as 12 glass drums consecutively. This permitted rapid accumulation of data, but left little time to make auxillary, but no less important, observations.

Two ideas presented in this paper ask further scrutiny. It appears that metallic ion toxicity as a function of micro-equivalents per liter gives percent "K_o" values quite different from those previously determined using mg/1 as the measure of concentration. A comprehensive study using

either conventional methods or the tracer methods discussed in this report might resolve these differences. The implications of the proposed definitions of synergistic and antagonistic effects need to be studied, discussed and extended.

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APPENDIX

"K" VALUES FOR VARIOUS BLANK RUNS

250 mg/1 Lactose		500 mg/1 Lactose	
"K"	γ[1]	"K"	γ
-0.0909[2]	-0.9943	-0.1163	-0.9922
-0.0968[2]	-0.9932	-0.1237[3]	-0.9977
-0.1060	-0.9953	-0.1286[3]	-0.9975
-0.1005	-0.9962	-0.1177[3]	-0.9947
-0.1045	-0.9966	-0.1146[3]	-0.9895
-0.0940	-0.9959	-0.1168	-0.9935
-0.1038	-0.9951	-0.1322	-0.9931
-0.0919	-0.9917	-0.1153	-0.9831
		-0.1362	-0.9978
Mean Value of "K"		Mean Value of "K"	
-0.0985	±0.0053	-0.1234	±0.0031

750 mg/1 Lactose		1000 mg/1 Lactose	
"K"	γ	"K"	γ
-0.1447[2]	-0.9999	-0.2917	-0.9987
-0.1432[2]	-0.9995	-0.2164	-0.9708
-0.1491	-0.9799	-0.2412	-0.9789
-0.1537	-0.9784	-0.2314	-0.9986
-0.1858	-0.9998	-0.2302	-0.9996
-0.1893	-0.9989	-0.2259	-0.9997
-0.1777	-0.9961	-0.2012	-0.9974
-0.1717	-0.9968	-0.2069	-0.9806
Mean Value of "K"		Mean Value of "K"	
-0.1580	±0.0203	-0.2222	±0.0136

[1] γ = Coefficient of correlation for "K".

[2] Pairs of drums with attached slimes grown in common nutrient broths and placed in common experimental broths (see pg. 28).

[3] Pairs of drums with attached slimes grown in common nutrient broth and tested in separate experimental broths (see pg. 28).

[4] 95 percent confidence interval.

"K" VALUES FOR VARIOUS RUNS USING CHROMATE AS THE TOXIC ION

Concentration of Chromate Ions		Run #1		Run #2		Run #3	
mg/l	µe/l	"K"	Y	"K"	Y	"K"	Y
0	0	-0.2917	-0.9987	-0.2164	-0.9708	-0.2412	-0.9789
2	2.31	-0.2917	-0.9965	-0.1757	-0.9959	-0.2088	-0.9909
4	4.61	-0.1874	-0.9909	-0.1623	-0.9912	-0.1723	-0.9952
6	6.92	-0.1676	-0.9966	-0.1257	-0.9904	-0.1519	-0.9995
8	9.22	-0.1305	-0.9915	-0.1134	-0.9180	-0.1153	-0.9895
10	11.53	-0.1022	-0.9793	-0.0819	-0.8216	-0.0964	-0.9803

PERCENT K_o FOR VARIOUS RUNS USING CHROMATE AS THE TOXIC ION

Concentration of Chromate Ions	Run #1		Run #2		Run #3	
mg/l	% K_o	Log % K_o	% K_o	Log % K_o	% K_o	Log % K_o
0	100.00	2.000	100.00	2.000	100.00	2.000
2	82.88	1.918	80.36	1.905	86.72	1.938
4	64.04	1.806	73.97	1.869	71.37	1.854
6	57.53	1.760	57.99	1.763	63.07	1.800
8	44.86	1.652	52.05	1.716	47.72	1.679
10	34.93	1.543	37.44	1.573	39.83	1.600
Slope $\pm$ 95% C.I.	-0.0452	±0.0080	-0.0408	±0.0128	-0.0425	±0.0068
Y	-0.9930		-0.9785		-0.9942	

"K" VALUES FOR VARIOUS RUNS USING NICKEL AS THE TOXIC ION

Concentration of Nickel Ions		Run #1		Run #2		Run #3	
mg/l	µe/l	"K"	γ	"K"	γ	"K"	γ
0	0	-0.2314	-0.9986	-0.2302	-0.9996	-0.2259	-0.9997
2	0.68	-0.1723	-0.9979	-0.1623	-0.9957	-0.1512	-0.9829
4	1.36	-0.1214	-0.9748	-0.1408	-0.9705	-0.0000	-0.0000
6	2.05	-0.0916	-0.7071	-0.1044	-0.9894	-0.1107	-0.9649
8	2.73	-0.0709	-0.9936	-0.0688	-0.9840	-0.0609	-0.9337
10	3.41	-0.0540	-0.9514	-0.0469	-0.7997	-0.0460	-0.9795

PERCENT K_o FOR VARIOUS RUNS USING NICKEL AS THE TOXIC ION

Concentration of Nickel Ions	Run #1		Run #2		Run #3	
mg/l	% K_o	Log % K_o	% K_o	Log % K_o	% K_o	Log % K_o
0	100.00	2.000	100.00	2.000	100.00	2.000
2	78.36	1.894	70.43	1.848	68.81	1.825
4	52.38	1.719	61.30	1.788	. .	. .
6	39.83	1.600	45.22	1.655	49.12	1.691
8	30.74	1.488	30.00	1.477	26.99	1.431
10	23.37	1.369	20.43	1.310	20.35	1.309
Slope ± 95% C.I.	-0.0641	±0.0088	-0.0693	±0.0174	-0.0666	±0.0362
γ	-0.9957		-0.9860		-0.9637	

"K" VALUES FOR VARIOUS RUNS USING COPPER AS THE TOXIC ION

Concentration of Copper Ions		Run #1		Run #2	
mg/l	µe/l	"K"	Y	"K"	Y
0	0	-0.2012	-0.9974	-0.2069	-0.9806
2	0.63	-0.0809	-0.9680	-0.0976	-0.9853
4	1.26	-0.0776	-0.9759	-0.0853	-0.9975
6	1.89	-0.0786	-0.9989	. .	. .
8	2.52	-0.0676	-0.9930	-0.0859	-0.9930
10	3.15	-0.0700	-0.9997	-0.0806	-0.9675

PERCENT K_o FOR VARIOUS RUNS USING COPPER AS THE TOXIC ION

Concentration of Copper Ions	Run #1		Run #2	
mg/l	% K_o	Log % K_o	% K_o	Log % K_o
0	100.00	2.000	100.00	2.000
2	40.30	1.605	47.34	1.675
4	38.81	1.589	41.06	1.613
6	39.30	1.594	. .	. .
8	33.83	1.529	41.55	1.619
10	34.83	1.542	39.13	1.592
Slope ± 95% C.I.	-0.0093	±0.0080	-0.0080	±0.0061
Y	-0.8666		-0.8276	

"K" VALUES FOR VARIOUS RUNS USING MIXTURES OF CHROMATE AND NICKEL IONS

Concentrations of Metallic Ions						Run #1		Run #2	
Chromate		Nickel		Total					
mg/l	µe/l	mg/l	µe/l	mg/l	µe/l	"K"	γ	"K"	γ
2	2.31	0	0	2	2.31	-0.1190	-0.9987	-0.2134	-0.9992
2	2.31	2	0.68	4	2.99	-0.0680	-0.7670	-0.1297	-0.9974
2	2.31	4	1.36	6	3.56	. .	. .	-0.0876	-0.9619
2	2.31	6	2.05	8	4.35	-0.0388	-0.9876	. .	. .
2	2.31	8	2.73	10	5.03	-0.0318	-0.8046	-0.0452	-0.9936
2	2.31	10	3.41	12	5.71	-0.0170	-0.6832	. .	. .

PERCENT K_o FOR VARIOUS RUNS USING MIXTURES OF CHROMATE AND NICKEL IONS

Concentrations of Metallic Ions			Run #1		Run #2	
Chromate mg/l	Nickel mg/l	Total mg/l	% K_o	Log % K_o	% K_o	Log % K_o
2	0	2	100.00	2.000	100.00	2.000
2	2	4	57.63	1.761	61.03	1.786
2	4	6	. .	. .	41.31	1.616
2	6	8	33.05	1.519	. .	. .
2	8	10	27.12	1.433	21.12	1.325
2	10	12	14.41	1.159	. .	. .
Slope ± 95% C.I.			-0.0708	±0.0329	-0.0762	±0.0094
γ			-0.9731		-0.9993	

"K" VALUES FOR VARIOUS RUNS USING CHROMATE AND NICKEL AND CHROMATE AND COPPER AS THE TOXIC IONS

Concentrations of Metallic Ions

Chromate		Nickel		Total		"K"	γ	% K_o
mg/1	µe/1	mg/1	µe/1	mg/1	µe/1			
4	4.61	0	0	4	4.61	-0.1740	-0.9924	100.00
4	4.61	6	2.05	10	6.66	-0.0833	-0.9967	47.88
4	4.61	8	2.73	12	7.34	-0.0817	-0.9911	45.98

Concentration of Metallic Ions

Chromate		Copper		Total		"K"	γ	% K_o
mg/1	µe/1	mg/1	µe/1	mg/1	µe/1			
2	2.31	0	0	2	2.31	-0.2295	-0.9972	100.00
2	2.31	4	1.26	6	3.59	-0.0898	-0.9989	39.17
2	2.31	10	3.15	12	5.46	-0.0903	-0.9986	39.30

Because of insufficient data the slope of the Log % K_o was not calculated for either of the runs above.